Dissipation of 17β-Estradiol in Composted Poultry Litter

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The excreted estrogen rate of all livestock in the United States is estimated at 134 kg d^{-1}. The influence of manure treatment on the fate of estrogens is critical in deciding the recycling of over 300 million dry tons of livestock produced annually. The effects of two common manure management practices, heated composting and ambient temperature decomposition, on the fate of 17β-estradiol in poultry litter were determined. A mixture of poultry litter, wood chips, and straw was amended with [^{14}C]17β-estradiol and allowed to undergo decomposition with a laboratory-scale heated composter (HC) or room temperature incubation (RTI) for 24 d. Radiolabel in the finished products was fractionated into water-extractable, acetone-extractable, nonextractable, and mineralized fractions. Total 17β-estradiol radioactive residues in the HC and RTI (n = 2) treatments were not different (P > 0.05), except that statistically less 17β-estradiol was mineralized to $^{14}CO_2$ during HC than RTI (1.1 vs. 10.0% for HC and RTI, respectively). Estrone was the major degradation product in extracts of HC and RTI treatments as determined by liquid chromatography/mass spectrometry analyses. The nonextractable residues indicated no quantitative differences among the humins between the treatments. An estimated 3% of the fortified estrogenicity remained after HC treatment, and 15% of the fortified estrogenicity remained after RTI treatment. If reduction of water-removable, biologically active 17β-estradiol is the treatment goal, then HC treatment would be slightly preferred over ambient temperature degradation. However, unmanaged, ambient temperature litter piles are less costly and time consuming for food animal producers and result in greater mineralization and similar immobilization of estradiol.

Endocrine-disrupting compounds (EDCs) interfere with the normal functioning of an organism's endocrine system (Lintelmann et al., 2003). Aquatic organisms can be readily affected by EDCs via surface water contamination (Routledge et al., 1998). Among the most potent EDCs are estrogens, androgens, and progestins (Lee et al., 2007), which are components of animal wastes. It is estimated that 304 million dry tons of animal wastes are produced annually in the United States from confined animal feeding operations (USDA Agricultural Research Service, 2006), which is over 100 times greater than the mass of human sewage sludge processed by municipal waste treatment facilities in the United States (Gerba and Smith, 2004). The excreted estrogen from all livestock in the United States is estimated at 134 kg d^{-1}, which is 2 to 3 times more than humans excrete in the United States (Lange et al., 2002). On the eastern shore of Maryland, 200,000 dry tons of broiler litter, containing 30 μg kg^{-1} 17β-estradiol, is produced annually, releasing about 6 kg of 17β-estradiol to the environment (Shore et al., 1995). The impacts of these hormones after manure application to croplands are potentially significant. Hormones in animal waste have been shown to affect groundwater quality because concentration trends for fecal coliforms and *Escherichia coli* with 17β-estradiol were positively correlated in a mantled karst system (Peterson et al., 2000).

17β-Estradiol has a water solubility of 3.9 mg L^{-1} (Hurwitz and Liu, 1977), and exogenous exposures in the micrograms per liter range have been shown to be associated with numerous pathologies in nontarget organisms. For example, Routledge et al. (1998) reported that exposure to 0.01 μg L^{-1} (10 ppt) 17β-estradiol (E2) for 3 wk significantly induced vitellogenin production in male rainbow trout (*Oncorhynchus mykiss*). Intermittent exposures (alternate days, 1 d in 4 or 3 d in 6) to 0.120 μg L^{-1} (120 ppt) E2 also resulted in significant vitellogenin induction in laboratory experiments using fathead minnows (*Pimephales promelas*) (Panter et al., 2000). Vitellogenin is an egg-yolk precursor protein normally produced by female fish.

The concern over EDCs found in animal manures has led to studies of decomposition and immobilization of these compounds in common farm disposal practices. Composting, whereby manures are stacked and turned occasionally to achieve thermophilic temperatures, decomposes organic matter to a stable,

J. Environ. Qual. 40:1560–1566 (2011)
doi:10.2134/jeq2010.0538
Posted online 8 July 2011.
Received 21 Dec. 2010.
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Abbreviations: EDC, endocrine disrupting compounds; HC, heated composter; LC/MS, liquid chromatography/mass spectrometry; LSC, liquid scintillation counter; RTI, room temperature incubation; TRR, total radioactive residues; WWTP, wastewater treatment plant.

humus-like material. Previous work has shown that water-soluble levels of 17β-estradiol and testosterone were decreased 84 and 90%, respectively, during aerobic composting of poultry litter in the field (Hakk et al., 2005). An alternative treatment method available to farmers is to move manures to storage areas where decomposition occurs at ambient temperatures. To determine the chemical fate of 17β-estradiol under common farm practices, laboratory studies using [^{14}C]17β-estradiol were conducted simulating aerobic, thermophilic composting and ambient temperature decomposition.

Materials and Methods

Materials and Chemicals

Separate laboratory-scale composting and ambient temperature studies were initiated with carbon-14 labeled 17β-estradiol in a poultry litter mixture. Fresh chicken layer litter was obtained from eight separate sites from a commercial producer in Lake Park, Minnesota. Litter samples were pooled, and aliquots of the homogenized litter were frozen at −28°C for subsequent nutrient analyses (Soil Testing Laboratory, North Dakota State University, Fargo, ND). The litter was mixed with pine chips and chopped straw (2:1:3 litter/wood/straw; v/v/v) to produce the starting poultry litter mixture, which had a carbon/nitrogen ratio of 31 on a dry mass basis. The poultry litter mixture was divided into four portions, two to be placed into self-heating laboratory-scale composters (HC #1 and #2) and two to be placed into incubators maintained at room temperature (RTI #1 and #2). Replicates of the HC 17β-estradiol mixture weighed 2.030 and 2.146 kg (HC #1 and #2, respectively), and the replicates of the RTI mixture weighed 0.703 and 0.789 kg (RTI#1 and #2, respectively). [4-^{14}C]17β-Estradiol, >98% radiolabeled purity by TLC (American Radiolabeled Chemicals, Inc., St. Louis, MO), was added evenly to the starting poultry litter mixture from a solution of absolute ethanol. For the HC study, 16.5 μg (2.7 μCi; average 7.9 ng g^{-1} compost) of [^{14}C]17β-estradiol were added (fresh weight basis). For the RTI portions of the study, 4.1 μg (0.68 μCi; average 5.5 ng g^{-1} compost) of [^{14}C]17β-estradiol were used.

Incubation Experiments

The litter mixture used for the HC treatment was placed into self-heating, laboratory-scale composters, which are described in detail by Sikora et al. (1983) and Arikan et al. (2007). The composters were double-walled with an internal screen basket, which contained the poultry litter mixture, and a stainless steel outer walled container, which provided an air-tight seal. Each composter was connected to a compressed air tank with an in-line bubble flow meter adjusted to maintain an airflow of approximately 30 mL min^{-1}. In preliminary experiments, this airflow rate was determined to maintain temperatures below 70°C. Water-saturated compressed air, produced as a result of bubbling through an in-line water trap, was introduced into one end of the composter through a gas-tight fitting. Through a second fitting, a thermocouple and a resistance temperature detector were inserted. Another set of temperature and resistance temperature detector probes was located in a water bath, which was maintained at 2°C below that of the heated compost using a controller. The thermocouples were connected to an OMEGA OM-220 data logger (Omega Corp., Stamford, CT). Temperature data were recorded every 15 min for 24 d.

An in-line C-18 SepPak cartridge (Waters, Milford, MA) was used to trap volatiles from compost gases. Carbon dioxide present in compost gas was trapped by bubbling the effluent through 250 mL of 3 mol L^{-1} NaOH. Aliquots of NaOH were assayed in triplicate using a liquid scintillation counter (LSC) (LS6000IC; Beckman Instruments, Inc., Fullerton, CA) with Ecolite scintillation cocktail (ICN, Costa Mesa, CA). The NaOH traps were changed promptly when the neutralizing capacity was consumed, as indicated by the precipitation of sodium bicarbonate/carbonate. The NaOH trapping solutions were titrated to measure total CO_2. One milliliter of NaOH sample was pipetted into a beaker along with phenolthalein indicator and titrated against standardized 0.2 mol L^{-1} HCl solution. Two RTI incubators were constructed by sandwiching a 17 × 18 cm (height × diameter) Pyrex jar (0.5 cm wall thickness) (Ace Glass, Inc., Vineland, NJ) between two rubber gasket–sealed aluminum plates secured at each corner with long bolts. Through a rubber stopper placed into a hole in the top aluminum plate, two glass tubes were inserted: one attached to an aquarium pump (Profile 1000; Meiko Pet Corp., Tai Chung, Taiwan; adjusted to 30 mL min^{-1} air flows with rotamers) with an in-line bubbler (Ace Glass) filled with water to introduce humidified air and the other attached to a bubble flowmeter, an in-line C-18 SepPak cartridge (Waters, Milford, MA), and a 250-mL 3 mol L^{-1} NaOH trap. Incubation temperature was monitored daily using a glass thermometer placed in the glass jar. The starting poultry litter mixtures were loosely packed into the RTI jar to allow for maximum air infiltration.

Analytical Methods

Aliquots of all starting poultry litter mixtures and finished products were freeze-dried to a constant weight and homogenized in a Waring blender. Triplicate subsamples (5 g each) were removed for extraction by water (3 × 100 mL) and acetone (3 × 100 mL). Nonextractable radioactivity was determined by combusting 0.1- to 0.3-g aliquots (n = 12) with a tissue oxidizer (Model 307; Packard, Meridan, CT).

Water and acetone extracts were concentrated by centrifugal rotary evaporation (Speed-Vac; Savant Instruments, Inc.; Holbrook, NY). [^{14}C]17β-Estradiol metabolites in the extracts were quantitated by fractionating the extracts with HPLC (Gilson System 45NC Gradient Analytical; Gilson Medical Electronics, Middleton, WI) into 1-mL effluents and counting 100-μL aliquots of the effluent fractions by LSC. High-performance liquid chromatography fractionation was accomplished in reversed-phase mode with a C18 Delta-Pak column (8 × 100 mm) (Waters, Milford, MA) under isocratic conditions (60:40 water/acetonitrile) with authentic [^{14}C]17β-estradiol and [^{14}C]estrone standards (American Radiolabeled Chemicals, Inc.). Endogenous 17β-estradiol present in the lyophilized, homogenized, starting manure mixture was quantified in an acetone extract, which was spiked with d_4–17β-estradiol internal standard (Sigma-Aldrich, St. Louis, MO) by liquid chromatography/mass spectrometry (LC/MS) against a six-point external standard curve of 17β-estradiol (5–200 pg 17β-estradiol on-column).

Distribution of nonextractable radioactivity into humic acid, fulvic acid, and humin components was determined using the methods of Kaplan and Kaplan (1982). Briefly, 2 × 2 g of nonextractable HC or RTI residue was rinsed with 25 mL of 0.1 mol L^{-1} HCl. The residue was extracted for 8 h with 20 mL of 0.5 mol L^{-1} NaOH, filtered, and extracted for 8 h with 35 mL 0.1 mol L^{-1} $Na_4P_2O_7$. After filtration, the two layers were combined and assayed for radioactivity by LSC. Three aliquots (0.1 g each) of the remaining insoluble residue (humin) were combusted with a tissue oxidizer (Model 307; Packard, Meridan, CT). After acidification (pH 3) of the NaOH/$Na_4P_2O_7$ layers with concentrated HCl, a precipitate formed (humic acid), which was separated by centrifugation (4303 × *g*), dried, and combusted as four aliquots in the tissue oxidizer. Aliquots of the soluble layer (fulvic acid) were assayed for radioactivity by LSC.

A Waters Quadrupole-Time of Flight mass spectrometer (Q-TOF Ultima API-US; Waters, Beverly, MA) equipped with an electrospray ionization source was used to characterize radiolabeled metabolites found in the water and acetone extracts of the finished product. Analyses were performed in negative-ion MS mode, and mass spectra of parent compound and metabolites took into consideration the two mass unit defects introduced by the >98% radiochemical purity C-4 atom. The capillary voltage was 2.33; the cone voltage was 55; source and desolvation temperatures were 120 and 400°C, respectively; and cone and desolvation gas flows (L h^{-1}) were 0 and 500, respectively. The LC system was an Alliance 2695 Separation Model (Waters, Beverly, MA) equipped with Symmetry C18 analytical (3.5 μm, 2.1 × 100 mm) and guard (2.1 × 10 mm) columns. The initial mobile phase was 60% 95:5 water/acetonitrile (solvent A) and 40% acetonitrile (solvent B) at a flow rate of 0.2 mL min^{-1}. A linear gradient to 100% solvent B was used from time 0 to 10 min, followed by a hold for 5 min.

Statistical differences between means of selected endpoints (mineralization or extractions) were determined using Student's *t* test (SigmaStat; Jandel Scientific Sorftware, San Rafael, CA).

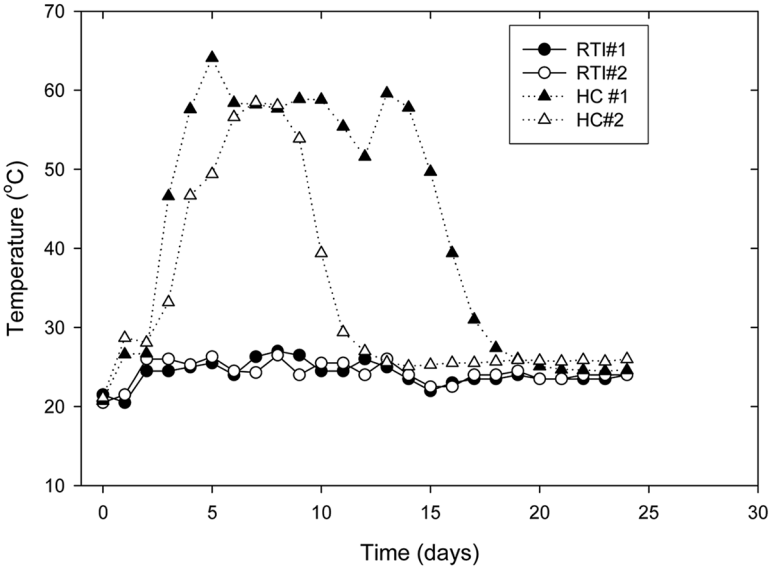


Fig. 1. Internal temperatures across time from laboratory-scale heated composting (HC) (*n* = 2) and room temperature incubation (RTI) (*n* = 2) for [^{14}C]17β-estradiol-spiked poultry litter/wood chipschopped straw (2:1:3 v/v/v).

Results

Decomposition during Heated Composter and Room Temperature Incubation Treatments

During the 24-d incubation, an average mass loss of 0.372 ± 0.023 kg was measured in HC amended with [^{14}C]17β-estradiol, representing about 18% of the starting mass (data not shown). Carbon dioxide trapped in the NaOH accounted for 0.178 ± 0.003 kg of the mass loss, and the remainder was water loss. Radioactivity was not detected in the in-line solid-phase extraction cartridges, indicating that volatile metabolites were not formed. Thermophilic temperatures (>40°C) in the HC composters were achieved in 3 to 4 d and were maintained for 6 to 13 d (Fig. 1). Thereafter, during the curing phase, mesophilic temperatures (<40°C) characterized the composting mass. Temperature rises in the duplicate self-heating composters were variable, probably due to the 3 or 4 airflow adjustments required to achieve and maintain thermophilic temperatures. Temperatures of the RTI incubators from both experiments did not vary by more than a few degrees from the ambient (range, 21–26°C) (Fig. 1).

Total recovery of 17β-estradiol TRR from CO_2, water, and acetone extracts and from nonextractables was 101% in HC and 111% in RTI treatments; therefore, recovery data were normalized to 100% for ease of comparison. Quantitative differences ($P > 0.05$) were not observed between the 17β-estradiol HC and RTI treatments for TRR in water and acetone extracts or for nonextractable TRR (Table 1).

Heated composting and room temperature incubation proceeded actively, as indicated by total CO_2 production from both study designs (Fig. 2). In the case of HC, total CO_2 production increased more quickly during the thermophilic stage, whereas in RTI decomposition, a constant production of CO_2 across the entire experiment was measured. Cumulative radiolabeled CO_2 output as a percentage of applied dose in HC was constant and very low (~1% of dose) during the 24 d (Fig. 3). For RTI, an initial induction period of approximately 8 d, where low [^{14}C]CO_2 output occurred, was followed by a rapid increase in [^{14}C]CO_2 evolution as a percentage of applied dose for the remainder of the study. The mean percentage of the radiolabel recovered as [^{14}C]CO_2 after [^{14}C]17β-estradiol amendment differed ($p = 0.0016$) between HC (1.1 ± 0.08%) and RTI (10.0 ± 0.4%) treatments.

Analyses of Extractable Products

High-performance liquid chromatography analyses of water and acetone extracts of HC and RTI with authentic standards suggested that parent compound and estrone (data not shown) were present. Chemical characterizations were confirmed by negative-ion LC/MS/MS with authentic standards (Table 2). Specifically, molecular ions for [^{14}C]17β-estradiol at m/z 273.17 [M-H] and for [^{14}C]estrone at m/z 271.16 [M-H] were present. Diagnostic fragment ions common to both hormones (m/z 185.08 and m/z 147.06) confirmed the identity of each compound. A polar metabolite of [^{14}C]17β-estradiol was also

Table 1. Fractionation of radioactivity of the finished products from heated composting or room temperature incubation of poultry litter mixture spiked with [^{14}C]17β-estradiol after 24 d (n = 2). [^{14}C]CO_2, water, and acetone-extractable and nonextractable data were normalized for total recovery.

	HC#1†	HC#2	Mean ± SD	RTI#1‡	RTI#2	Mean ± SD
	% dose			% dose		
[^{14}C]CO_2	1.1	1.0	1.1 ± 0.08*	9.7	10.3	10.0 ± 0.4*
H_2O extract	28.9	27.0	27.9 ± 1.3	30.5	30.0	30.2 ± 0.4
Acetone extractable	47.0	43.0	45.0 ± 2.8	40.6	42.5	41.6 ± 1.3
Nonextractable	23.0	29.0	26.0 ± 4.2	19.2	17.3	18.2 ± 1.4
Humic substances§						
Fulvic acid	23.3	24.2	23.8 ± 0.7	27.7	30.8	29.2 ± 2.2
Humic acid	23.8	38.3	31.0 ± 10.3	39.5	33.3	36.4 ± 4.4
Humin	52.9	37.4	45.2 ± 11.0	32.8	35.9	34.3 ± 2.2

* Means were statistically different (P > 0.05) between heated composting and room temperature incubation compartments.

† HC, heated composting.

‡ RTI, room temperature incubation.

§ Humic substances are expressed as fractional percentages of the nonextractables and were normalized.

present in the HPLC chromatograms of water and acetone extracts of HC and RTI finished product. The metabolite had a retention time on LC/MS identical to standard estriol and a molecular ion at 289.18, but, with very weak fragments at m/z 147.06 and 173.08 and with no diagnostic m/z 185.08, it remains unidentified. Only 3% (HC) or 15% (RTI) of the initial [^{14}C]17β-estradiol was present in combined water and acetone extracts after 24 d (Table 2). Forty-eight percent (HC) and 57% (RTI) of the applied dose was recovered as estrone in water and acetone extracts after 24 d. The unidentified polar metabolites constituted 21 and 2% of the combined extracts, respectively.

Analysis of Nonextractable Products

Total recovery of radioactivity into fulvic acid, humic acid, or humin fractions after the partitioning of the nonextractable residues ranged from 92 to 113%. Therefore, the data were normalized for recovery. Quantitative differences were not observed for any fraction between HC and RTI (P > 0.05) (Table 1). Radiolabel derived from [^{14}C]17β-estradiol partitioned least into fulvic acid for HC and RTI treatments and most into humin (Table 1).

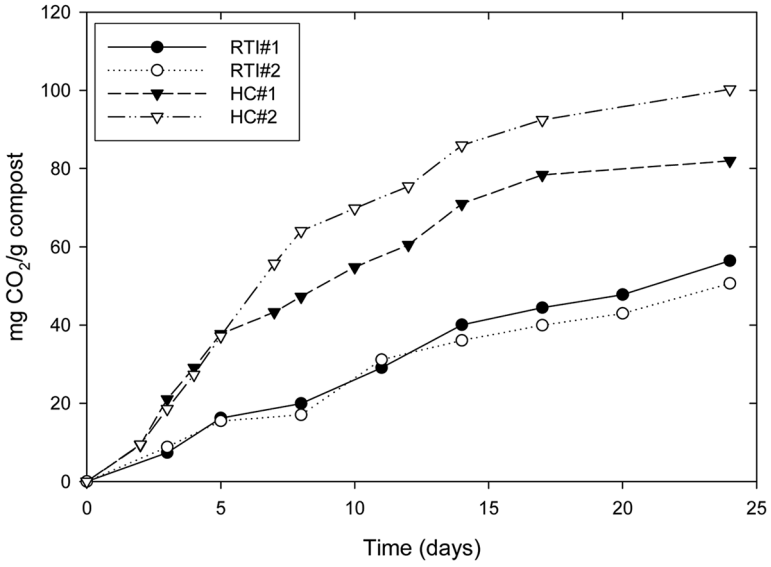


Fig. 2. Cumulative evolution of total CO_2 per gram of starting poultry litter mixture during the heated composting (triangles) or ambient temperature decomposition (circles) of [^{14}C]17β-estradiol–amended chicken litter, straw, and wood chips (2:3:1). Each duplicate experiment is presented separately. HC, heated composting; RTI, room temperature incubation.

Discussion

Temperatures

Microorganisms are responsible for the biological degradation of organic matter during aerobic composting and during incubations conducted at ambient temperatures (Arikan et al., 2007). Microorganisms consume digestible organic matter and O_2 and generate stable organic materials, H_2O, CO_2, and heat as by-products (Dougherty, 1999). The microorganisms present in the poultry litter of this study were not characterized, but previous research has shown that chicken layer manure consists of a variety of beneficial bacteria, actinomycetes, fungi, algae, and protozoa (Dougherty, 1999). Increasing the temperatures during composting affects the type and proportion of these microorganisms. Biological activity often is correlated with temperature, but microbial populations and available carbon in the mixture also influence activity. Temperature increases within the mesophilic range lead to increased biological activity, but when temperatures reach the thermophilic range, increased biological activity may not be sustained. A benefit of composting manure or biosolid mixtures at temperatures above 50°C is the reduction or elimination of labile human and plant pathogens (Sikora, 1998).

The ambient temperatures in the RTI experiments would presumably activate a microorganism population different from that found during the thermophilic stages of HC. Many variables control aerobic composting, such as carbon/nitrogen ratio, moisture content, porosity, and particle size (Dougherty, 1999). Because of the methods and feedstock, the results of this study may not be representative of other reports of poultry waste remediation. Therefore, the present results should be understood in terms of the conditions and constraints selected.

In this study, thermophilic temperatures in HC were achieved 1 to 4 d after initiation of composting

(Fig. 1). Return to mesophilic temperatures by 16 d was an indication that biological activity had diminished and that the curing phase had begun. The experiments were halted within 6 to 12 d. Temperature differences among replicates were probably due to difficulty in establishing identical airflows between composters and in achieving an identical degree of bulkiness and porosity inside the composters.

Hormone Fate

The baseline concentration of 17β-estradiol in the acetone extracts of starting manure mixture used for the HC and RTI treatments was 220 ng g^{-1}. Taking into account dilution with wood chips and chopped straw used to make the treated compost, the baseline 17β-estradiol concentration was within the 14 to 904 ng g^{-1} (dw) range previously reported for poultry litter (Hanselman et al., 2003; Shore et al., 1993).

The immediate generation of CO_2 from both study designs was indicative of properly working degradation systems (Fig. 2). The general shape of the cumulative, total CO_2 curves in the HC treatments consisted of rapid CO_2 increases during the thermophilic phase and a reduction in CO_2 production once the curing phase was initiated. These data are typical of self-heating laboratory composters (Arikan et al., 2007; Sikora et al., 1983) where rapid degradation occurs early but declines with mesophilic conditions. In the RTI replicates, cumulative carbon dioxide curves were linear throughout the study period, suggesting that constant degradation of organic matter occurred at ambient temperatures.

Generation of [^{14}C]CO_2 from each treatment provided unambiguous evidence that the fate of 17β-estradiol included, at least in part, complete oxidation. The temporal pattern of [^{14}C]CO_2 release also indicated that degradation of 17β-estradiol to CO_2 occurred with thermophilic and mesophilic microorganisms (Fig. 3). Radiolabeled CO_2 production was statistically greater under RTI than under HC conditions (P = 0.0016), suggesting that mineralization of 17β-estradiol was a more favored pathway for mesophiles compared with thermophiles. Quantitative differences, as well as different pathways, in the degradation of organic compounds undergoing mesophilic degradation or thermophilic composting have been observed previously (Finstein and Morris, 1975).

[^{14}C]17β-Estradiol was labeled on the C-4 position of the A-ring; therefore, [^{14}C]CO_2 could only result from the complete oxidation of this aromatic ring. Hemmings and Hartel (2006) showed that mineralization of 17β-estradiol could occur in poultry litter and that the extent of mineralization was directly related to water content but inversely related to temperature. Less than 2% of applied 17β-estradiol was mineralized at 35 and 45°C under constant water content, whereas 5.9% of the applied 17β-estradiol was mineralized at 25°C (Hemmings and Hartel, 2006). These data support the observation of a statistically greater mineralization of 17β-estradiol during RTI than HC conditions (Table 1). Soil microorganisms are also capable of mineralizing 17β-estradiol. For example, Colucci et al. (2001) reported that 11 to 17% of a 1 mg kg^{-1} dose of 17β-estradiol in soil was mineralized in 91 d. Similarly, 6% of [^{14}C]17β-estradiol was mineralized after 5 d of incubation in a water-saturated, sandy-loam soil (Fan et al., 2007).

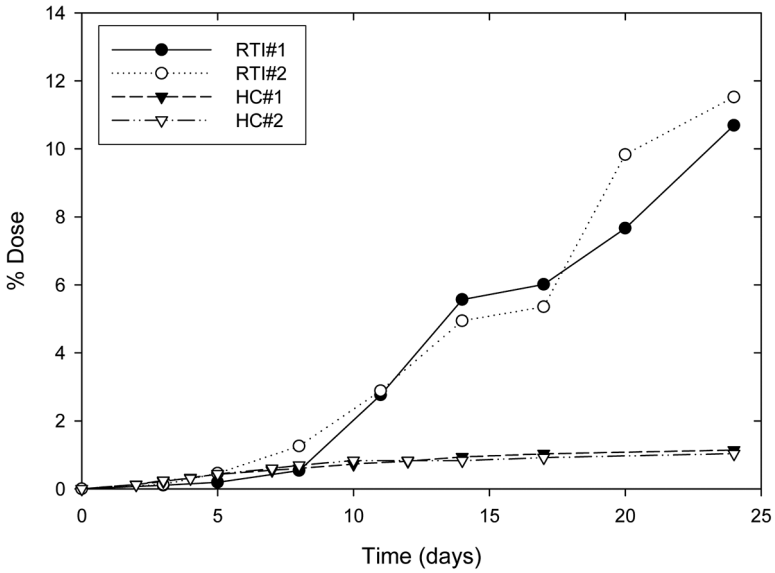
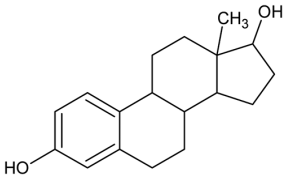
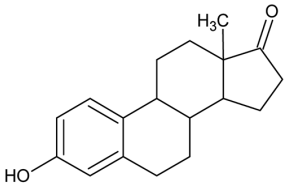


Fig. 3. Cumulative evolution of [^{14}C]CO_2 per gram of starting poultry litter mixture during the heated composting (triangles) or ambient temperature decomposition (circles) of [^{14}C]17β-estradiol amended chicken litter, straw, and wood chips (2:3:1). Each duplicate experiment is presented separately. HC, heated composting; RTI, room temperature incubation.

Approximately 30% of the applied radioactivity of 17β-estradiol was present in water extracts; however, a greater amount of TRR was observed for acetone extractables of both treatments (Table 1). Steroid hormones extractable into water would be readily mobile in the field, whereas acetone extractable hormones would be potentially mobile. Characterization of the constituents from combined water and acetone extracts of both treatments indicated that approximately 50% of the 17β-estradiol dose was metabolized to free estrone and only 3 or 15% of the applied dose could be accounted for as parent compound

Table 2. Quantitation of constituents detected in combined water and acetone extracts for [^{14}C]17β-estradiol amended into starting poultry litter mixture and allowed to undergo heated composting or room temperature incubation for 24 d.

Metabolite	HC†	RTI‡
	% dose	
 [^{14}C]17β-estradiol	3	15
 [^{14}C]estrone	48	57
Unknown polar metabolite	21	2

† Heated composting.

‡ Room temperature incubation.

after 24 d in HC and RTI treatments, respectively (Table 2). The present data showing a decline in extractable 17β-estradiol are in general agreement with the results of a 139-d windrow composting study with poultry litter where 84% of the water/acetone-extractable 17β-estradiol was degraded (Hakk et al., 2005). The ELISA analyses performed did not detect products such as estrone (Hakk et al., 2005). Zheng et al. (2008) demonstrated with dairy waste that estradiol levels declined by 1 to 3 orders of magnitude during the piling of dairy manure solids or in a typical dairy waste disposal system. The decline of 17β- and 17α-estradiol in these treatment systems was accompanied by an increase in estrone concentrations, suggesting that both stereoisomers of estradiol can be oxidized to estrone by dairy manure microorganisms (Zheng et al., 2008). Derby et al. (2010) reported little change in initial 17β-estradiol levels (0.64 μg kg^{-1}) at multiple time points across 92 d of composting swine manure. Estrone was present at far higher levels initially (29.4 μg kg^{-1}) in the swine manure and declined by 94% to 1.9 μg kg^{-1} after composting. A polar metabolite comprised 21 and 2% of the combined extracts in HC and RTI treatments (Table 2). Although this metabolite remains unidentified, its polarity and fragmentary LC/MS data suggest it could be estriol, which has been observed in poultry primary lagoons at levels higher than 17β-estradiol (Hutchins et al., 2007).

The oxidation of 17β-estradiol to estrone is a common event in the environment. Colucci et al. (2001) demonstrated that the oxidation of 17β-estradiol to estrone readily occurred under sterile and nonsterile soil conditions. Such oxidation has recently been shown to be mediated by manganese oxide (MnO), which is present in most soils (Sheng et al., 2009). Nutrient analyses of the poultry litter used for our experiments indicated that it contained >470 mg kg^{-1} of elemental Mn; however, MnO levels were not determined (Table 3). Estrone is one one-hundreth as active as 17β-estradiol, as determined by E-Screen assay (Soto et al., 1995). Assuming a relative estrogenicity of 1.0 for 17β-estradiol and 0.01 for estrone, an estimated 3% of the fortified estrogenicity remained after HC treatment, and 15% of the fortified estrogenicity remained after RTI treatment. Therefore, from an environmental perspective, heated composting of poultry litter would result in a marginal improvement over static pile incubation for estrogenicity reduction. Previous research with swine manure has demonstrated 79 and 74% reductions in original estrogenicity after 92 d with swine manure that was composted or held in a static pile, respectively (Derby et al., 2010).

Table 3. Characterization of the starting poultry litter used in the 17β-estradiol degradation experiments. The same litter was used for heated composter (*n* = 2) and room temperature incubation (*n* = 2) treatments. The starting mixture for heated composting or room temperature incubation was produced by mixing poultry litter with pine chips and chopped straw in a ratio of 2:1:3 (v:v:v). Data have been corrected to a dry matter basis.

Analysis	Poultry litter
Moisture content, %	72.9
N, g kg^{-1}	57.2
P_2O_5, g kg^{-1}	30.7
K_2O, g kg^{-1}	28.5
NH_4–N, mg kg^{-1}	7324
NO_3–N, mg kg^{-1}	1037
Ca, g kg^{-1}	98.9
Mg, mg kg^{-1}	6171
Zn, mg kg^{-1}	360
Cu, mg kg^{-1}	30.4
Fe, mg kg^{-1}	254
Mn, mg kg^{-1}	471
Conductivity, dS m^{-1}	8.4

Degradation of organic compounds during composting consists of two phases (Dougherty, 1999). The first phase is the degradation of large, complex molecules into smaller, building block compounds. During the second phase of composting (the curing phase during which humification occurs), organic residues become incorporated into humic substances (i.e., humic acid, fulvic acid, or humin). Humic substances are complex aromatic macromolecules containing amino acids, amino sugars, peptide, and aliphatic monomeric units. Humic acids are acid insoluble but are soluble at pH >8 (Kaplan and Kaplan, 1982). Fulvic acids are lower molecular weight than humic acids, are water-soluble regardless of pH, and contain a higher proportion of oxygen-bearing polar functional groups than humic acids. Humin is that portion of compost or soil that is insoluble at all pH levels and has a very high molecular weight (~300,000). Hydroxylation of composted xenobiotic chemicals followed by covalent coupling of the xenobiotics to humic and fulvic acids in soil has been demonstrated for pesticides, mineral oils, and explosives (Richnow et al., 1994; Calderbank, 1989; Thorn et al., 2002). When bound to humic substances, toxic xenobiotics tend to lose their inherent biological activity (Calderbank, 1989), which is a fundamental goal for successful remediation efforts. Long-term experiments are needed to determine if 17β-estradiol nonextractables in poultry litter could become biologically active again. In addition to humification, nonextractable residues may arise from adsorption (Berry and Boyd, 1985), ionic binding (Gevao et al., 2000), covalent binding (Bollag and Myers, 1992), or sequestration to compost or soil macromolecules (Pignatello and Xing, 1996). Depending on the nature of the nonextractable binding (i.e., physical or chemical), bound residues could be slowly released during the course of humic turnover (Mahro and Kästner, 1993). A method that may initially appear to be suitable for bioremediation may be a potential hazard for the environment in the future.

Summary and Conclusions

Heated composting and ambient temperature degradation of a poultry litter mixture resulted in the partitioning of [^{14}C]17β-estradiol TRR into four distinct compartments. Statistically significant differences in the amount of [^{14}C]CO_2 production were observed between HC and RTI study designs, but no statistical differences were observed in the extractable and nonextractable compartments. Estrone was the predominant byproduct. In each treatment, at least 85% of the 17β-estradiol was mineralized, degraded to metabolites, or incorporated into humic substances. Therefore, the data indicated that if the final endpoint of a poultry litter management plan is to reduce the water (rainfall) removable, biologically active 17β-estradiol, then HC treatment would be slightly preferred over ambient temperature degradation. Furthermore, if remobilization of bound steroid residues could be discounted, then aerobic com-

posting would clearly be the more environmentally desirable method for treating poultry manures versus storage at ambient temperature because of the additional benefits of composting, including moisture reduction, killing of pathogenic organisms, odor control, and possible production of a value-added product for the producer. However, if labor and equipment costs are an important consideration, then storage of poultry litter at ambient temperature may be a preferred method for producers because it requires less materials handling than composting but results in similar outcomes with regard to a decrease in estrogenicity and mobility of estrogens into the environment.

Acknowledgments

The authors thank Colleen Pfaff for technical assistance and Grant Harrington for LC/MS analyses. The use of trade, firm, or corporation names in this publication is for the information and convenience of the reader. Such use does not constitute an official endorsement or approval by the United States Department of Agriculture or the Agricultural Research Service of any product or service to the exclusion of others that may be suitable.

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